AWARD NUMBER: W81XWH-15-1-0543

TITLE: Disruption of the Interaction of the Androgen Receptor with Chromatin: A Novel Therapeutic Approach in Prostate Cancer

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REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
                            Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2016	Annual	8 Sep 2015 - 7 Sep 2016

4. TITLE AND SUBTITLE

Disruption of the Interaction of the Androgen Receptor with Chromatin: A Novel Therapeutic Approach in Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0543

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We have made significant progress in our work with small molecules including peptides/peptidomimetics to disrupt the interaction between androgen receptor and critical coregulators, such as the pioneering transcription factor FOXA1 on the DNA. We have shown that the interface between AR-FOXA1 plays important roles in AR responsiveness and activity. We have designed, synthesized, and tested a panel of peptides/peptidomimetics to disrupt the interaction between AR and FOXA1. The effects of these candidate compounds have been tested in multiple assays and various prostate cancer cell lines. We have further utilized and refined the potent and sensitive NanoBit *in vivo* complementation assay to evaluate other possible reagents through high throuput screening strategy. We are now in the process of comparing and confirming these lead compounds in prostate cancer models. In the coming year, we intend to further optimize these compounds and complete the proposed mechanistic studies.

15. SUBJECT TERMS
None provided

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE	Unclassified	9	19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified			

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INTRODUCTION:

For patients with metastatic PCa, the primary therapeutic modalities target the androgen receptor (AR), which is the key molecular driver of disease. While most men will respond to AR-targeted treatments, cancer often recurs with progression to castration-resistant PCa (CRPC). More potent second-generation AR-targeting agents, including abiraterone and enzalutamide, have shown efficacy against metastatic CRPC. However, durable responses to these newer agents are rare, with many men exhibiting de novo resistance, and all tumors will progress despite treatment. Our overarching hypothesis is that the disruption of interaction between AR and critical cofactors, such as the pioneering transcription factor FOXA1 on the DNA, would selectively disrupt AR signaling and effectively block CRPC growth. Aim 1: Select and evaluate peptides/peptidomimetics in models of PCa. Aim 2: Determine the molecular action of peptide /peptidomimetics at the chromatin level.

KEYWORDS:

Prostate cancer, Androgen Receptor, FOXA1, androgen receptor co-regulators, Protein-protein interactions, peptides/peptidomimetics

ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1: Select and evaluate peptides/peptidomimetics in models of PCa. Based on the modeled AR/FoxA1 interface, some candidate peptides/peptidomimetics will be rationally designed and synthesized by our collaborators. Each agent will be tested for its stability, pharmacologic properties, toxicity, efficacy in disrupting AR/FoxA1 PPI, and for activity on AR-driven gene expression. The most effective peptides/peptidomimetics, the drugs will be further validated in PCa models, including in multiple PCa cell lines *in vitro* and against C4-2B and CWR22rv1 xenografts *in vivo*.

Task 1: Select and evaluate peptides/peptidomimetics in vitro and validate the lead peptidomimetics *in vivo*.

- Based on the proposed AR/FOXA1 interface, we synthesized, and evaluated several important amino acid mutants within the interface and confirmed the importance of these amino acids in AR signaling activation within cell line models in vitro.
- We then synthesized multiple peptides from the proposed interaction interface and examined the effects of these peptides in the disruption of endogenous AR-FOXA1 complexes *in vivo*. We will continue to synthesize smaller peptides derived from this interaction motif.
- We developed an advanced NanoBiT *in vivo* complementation assay to study the interaction between overexpressed AR and FOXA1. This assay system facilitates the validation experiments of peptides/peptidomimetics.

Task 2: Evaluate the lead peptides/peptidomimetics in models of prostate cancer.

- We evaluated several peptides in multiple PCa cell line models to compare their therapeutic efficacies.
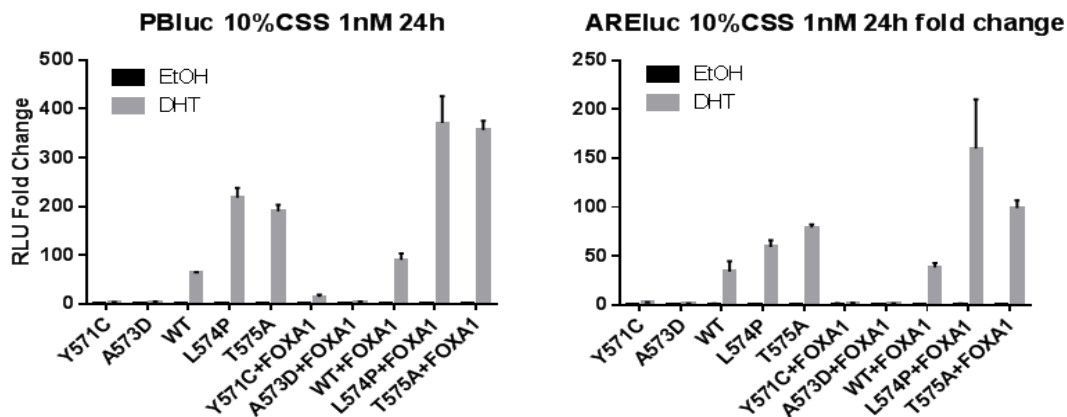
Aim 2: Determine the molecular activity of the peptides /peptidomimetics at the chromatin level. The effect of the peptide/peptidomimetics on AR/FoxA1 promoter occupancy will be assayed in basal and androgen-induced states using ChIP assays for individual promoters and ChIP-Seq for global promoter analyses.

Task 3: Investigate the molecular mechanisms of the peptides/peptidomimetics at the chromatin level
- We have optimized protocols for ChIP, reChIP, ChIP-Seq and ChIP-reChIP sequencing experiments including library preparation.
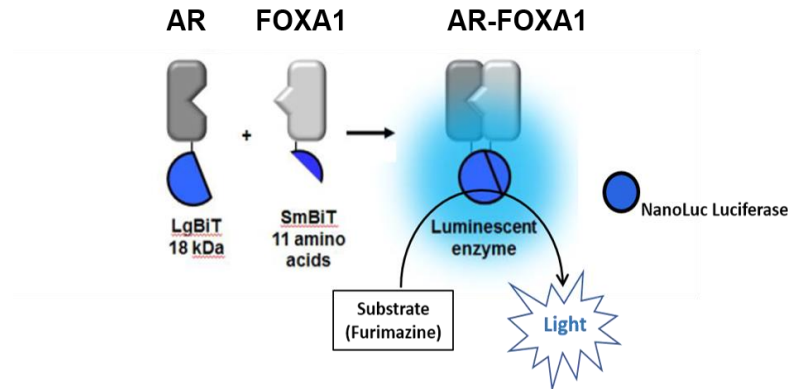
What was accomplished under these goals?

In this grant, we had proposed to design and synthesize peptides/peptidomimetics targeting structural motifs involved in AR-FOXA1 protein-protein interactions (PPIs). The interface provided in the preliminary data was based on computational modeling. We first had to validate the accuracy and potential function of this special interface in the biological systems. The predicted AR/FoxA1 interface consists of specific residues of human AR and specific residues of human FoxA1. Within the seven a.a interface on AR, point mutations of two a.a. occur in CRPC and point mutations of two a.a. occur in androgen insensitivity syndrome
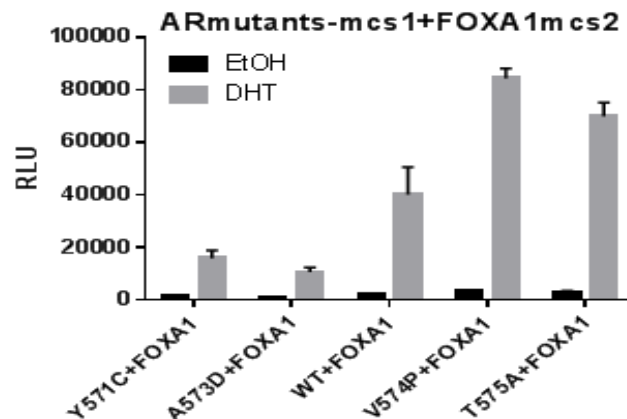

We wanted to identify the specific molecular interface within the interaction complex and to evaluate the effects of specific lead peptides/peptidomimetics in blocking AR signaling in various prostate cancer models. Further, we wanted to determine the molecular action of peptide /peptidomimetics at the chromatin level. In our first year, we have made significant progress in these plans. We have validated the PPI interface between AR-FOXA1, narrowed down a few important amino acids related to AR signaling activation. AR cDNAs carrying above four known AR mutants and wild-type AR cDNA were subcloned into expression vectors. To compare the mutants effects on AR activation by measuring DHT-induced Probasin (PB) promoter or ARE reporter activities, these five different constructs were cotransfected with or without FOXA1. Both PB and ARE activities were more significantly induced by DHT in CRPC mutants than the wildtype. In the presence of the FOXA1 coexpression, DHT-induced PB and ARE were further increased versus the vector control groups.
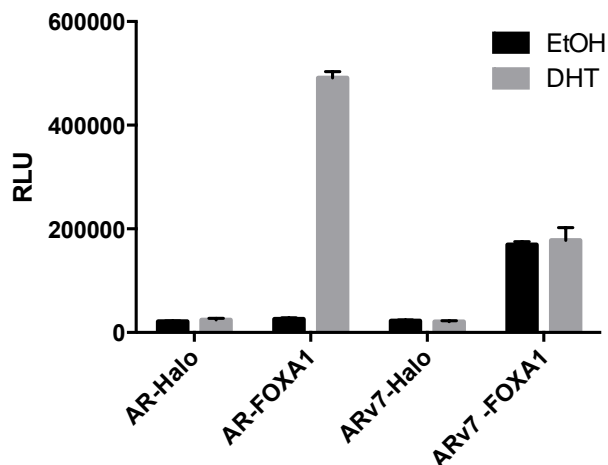
We have created and tested small peptide derived from this motif to examine their therapeutic efficacies. Additionally, we have integrated a cutting edge NanoBiT complementation assay to study the critical interaction between AR and FOXA1.

We have successfully fused AR wild-type and above four mutants cDNA to the LargeBit subunit of NanoBit enzyme and also fused FOXA1 cDNA to the SmallBit subunit of this enzyme. In cells expressing both proteins, the interaction between the AR-FOXA1 would bring the NanoLuc subunits in close proximity and catalyze the enzyme reaction in the presence of substrate Furimazine. The interaction can be detected and measured by luminescence.
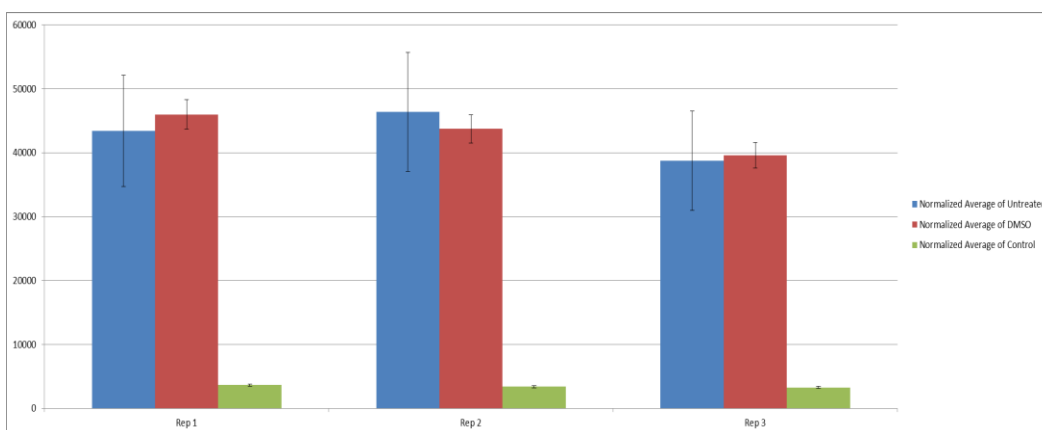

We cotransfected these recombinant ARs together with fused FOXA1 plasmids into HEK293T cells and stimulated the interaction by DHT for 30mins. From the NanoBit assay results, we found that DHT significantly increased the interaction between various ARs and FOXA1. The DHT-induced interaction was more in CRPC mutants than wild-type and AIS mutants.

The induction of constitutively active AR variants (AR-Vs) that lack the canonical ligand binding domain (LBD) is closely correlated with CRPC development and treatment resistance. Among AR-Vs, AR-V7 generated by alternative splicing is by far the most characterized variant regarding its expression, function, and clinical correlation with CRPC progression. Through our newly developed NanoBit assay, we found that ARv7 interacted with FOXA1 intrinsically, and the interaction was independent of DHT stimulation.

Besides testing the therapeutic effects of rationally design peptides/peptidomimetics as proposed in Aim I, we will also incorporate the potent NanoBit assay to evaluate other possible agents.

Currently, we have selected the ARv7-FOXA1 complex for HTS experiments and completed the HTS system optimization processes. As shown in our mock HTS experiments, ARv7-FOXA1 complex exhibited consistent and reproducible signal induction. The developed protocol with satisfactory screening window has z score=0.598 > 0.4.

We will utilize the UTSW 8k compound library to select those candidates which can disrupt ARv7-FOXA1 complex. By combining our lead peptide/peptidomimetics with these unbiasedly selected small molecules, we are going to test the effects of these compounds in various PCa models.

What opportunities for training and professional development has the project provided?

The training and professional development are important components required by DoD Postdoctoral Training Award. **Training**: PI has completed a series courses (3 credits per semester) provided by UTSW and received the postdoctoral certificate in Basic Research. **Professional development**: PI attended Gordon Research Conference on Hormone-Dependent Cancers in 2015.

How were the results disseminated to communities of interest?

Nothing to Report, as these are ongoing projects.

What do you plan to do during the next reporting period to accomplish the goals?

We have synthesized and tested a panel of peptides/peptidomimetics to disrupt the interaction between AR and FOXA1. The effects of these candidate compounds have been tested in multiple assays and multiple prostate cancer cell lines. We are going to get the screening results within a month. The successful hits selected from 8k library will be further compared with our lead peptides/peptidomimetics to pick the top candidates for the *in vivo* experiments and mechanistic studies.

Given that the small molecules identified from screening will be specific for blocking the interaction between AR splicing variant V7 and FOXA1, the candidate compounds have a significant impact in targeting AR variants.

We are about two months behind schedule regarding *in vivo* experiments due to the HTS setup and screening optimization. We believe that our potential hits from HTS will be a great addition to our rational designed peptides/peptidomimetics. Since we have learned and practiced the techniques required in Aim 2, we will move on this part more efficiently after confirming the efficacy of the lead compound *in vivo*.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We have shown that the interface between AR-FOXA1 plays important roles in AR responsiveness and activity by cotransfecting various AR mutants with or without FOXA1. These results confirm the validity of our proposed interaction model.

We have detected and quantified the interaction between AR/ARv7 and FOXA1 with the utilization of potent and sensitive NanoBit complementation assay. The impact of this novel assay is that more AR coregulators can be studied and targeted in a similar manner.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

CHANGES/PROBLEMS:

Nothing to report

PRODUCTS:

PUBLICATIONS, ABSTRACTS, AND PRESENTATION

1. Miao L, Yang L, Rodrigues D, Crespo M, Hsieh JT, Tilley WD, de Bono J, Selth LA, Raj GV. (2016) Disruption of androgen receptor signaling induces Snail-mediated epithelial-mesenchymal plasticity of prostate cancer. Cancer Res. (submitted)

2. Abstract: Miao L, Yang L, Tilley WD, de Bono J, Selth LA, Raj GV. (2015) Rapid Induction of mesenchymal phenotype in prostate cancer upon blockade of androgen receptor signaling. Gordon Research Conference on Hormone-Dependent Cancers. Newry, Maine.

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No Change